

FORM PTO-1390 (Modified)
(REV 11-2000)

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

**TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371**

ATTORNEY'S DOCKET NUMBER

100725-28 / Kreisler 1101-KGB

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR

10/070966

INTERNATIONAL APPLICATION NO.

PCT/EP00/08929

INTERNATIONAL FILING DATE

13 September 2000 (13.09.00)

PRIORITY DATE CLAIMED

13 September 1999 (13.09.99)

TITLE OF INVENTION

BIOREACTOR FOR FERMENTING SOLIDS

APPLICANT(S) FOR DO/EO/US

UDO HOLKER

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (24) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
 - a. ☐ is attached hereto (required only if not communicated by the International Bureau).
 - b. ☒ has been communicated by the International Bureau.
 - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
 - a. ☒ is attached hereto.
 - b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
 - a. ☐ are attached hereto (required only if not communicated by the International Bureau).
 - b. ☒ have been communicated by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☐ have not been made and will not be made.
8. ☒ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☒ Certificate of Mailing by Express Mail
23. ☒ Other items or information:

First Page of the application as published (WO 01/19954 A1)

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Seven Sheets of Drawings

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 1.492 (a) (1) - (5)) : 10/070966		INTERNATIONAL APPLICATION NO. PCT/EP00/08929		ATTORNEY'S DOCKET NUMBER 100725-28 / Kreisler	
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24. The following fees are submitted:.				CALCULATIONS PTO USE ONLY	
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) : <input type="checkbox"/> Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO \$1040.00 <input checked="" type="checkbox"/> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO \$890.00 <input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$740.00 <input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) \$710.00 <input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) \$100.00 <p style="text-align: center;">ENTER APPROPRIATE BASIC FEE AMOUNT =</p>				\$890.00	
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492 (e)). <p style="text-align: center;">\$0.00</p>					
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	26 - 20 =	6	x \$18.00	\$108.00	
Independent claims	2 - 3 =	0	x \$84.00	\$0.00	
Multiple Dependent Claims (check if applicable).				<input type="checkbox"/> \$0.00	
TOTAL OF ABOVE CALCULATIONS =				\$998.00	
<input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27). The fees indicated above are reduced by 1/2.				\$0.00	
SUBTOTAL =				\$998.00	
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492 (f)).				\$0.00	
TOTAL NATIONAL FEE =				\$998.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) (check if applicable).				<input type="checkbox"/> \$0.00	
TOTAL FEES ENCLOSED =				\$998.00	
				Amount to be: refunded	\$
				charged	\$

a. ☐ A check in the amount of _____ to cover the above fees is enclosed.

b. ☒ Please charge my Deposit Account No. **14-1263** in the amount of **\$998.00** to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **14-1263** A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Norris McLaughlin & Marcus
 220 East 42nd Street
 30th Floor
 New York, New York 10017
 Tel.: (212) 808-0700
 Fax: (212) 808-0844

SIGNATURE

Kurt G. Briscoe

NAME

33,141

REGISTRATION NUMBER

March 13, 2002

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Kreisler 1101-KGB
020451us/JH/ml

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS : UDO HÖLKER
SERIAL NO. : TO BE ASSIGNED
FILED : HEREWITH
FOR : BIOREACTOR FOR FERMENTING SOLIDS
ART UNIT : TO BE ASSIGNED
EXAMINER : TO BE ASSIGNED

March 13, 2002

Hon. Commissioner of Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

SIR:

Prior to examination, please amend the above-identified application as follows:

IN THE SPECIFICATION:

Insert as the first paragraph of the specification the following new paragraph: -- This application is a 371 of PCT/EP00/08929 filed on September 13, 2000. --

IN THE CLAIMS:

Cancel all of the claims in the application and substitute the following new claims:

UDO HÖLKER
KREISLER 1101-KGB

26. A bioreactor for fermenting solid substrates, comprising a fermentation vessel (2), a charging means for bioreactive substances, and a nozzle arrangement within said fermentation vessel (2) for aeration and thorough mixing of the substrates, wherein the at least one nozzle arrangement (10, 20) has a multitude of pipes (14, 24) extending in parallel into the reaction space (49) of the fermentation vessel (2) and provided with nozzles (16, 28), wherein a first, vertically extending nozzle arrangement (10) can be extended into and retracted from the reaction space (49) of the fermentation vessel (2), characterized by a second, horizontal nozzle arrangement with at least one pipe having nozzle orifices and extending horizontally through the reaction space.

27. The bioreactor according to claim 26, wherein a second, horizontal nozzle arrangement (20) is provided which consists of at least two interconnected pipes (24) extending horizontally and in parallel through the reaction space (49), each having a plurality of nozzle orifices (28).

28. The bioreactor according to claim 26, wherein said horizontal nozzle arrangement (20) can be rotated around a horizontal rotation axis.

29. The bioreactor according to claim 26, wherein said fermentation vessel (2) has a bottom section (32) with a tapering cross-section.

30. The bioreactor according to claim 29, wherein said conically designed bottom section (32) leads into a draining channel (36) which is inclined from horizontal and has a draining valve (40) at the lowest position thereof.

31. The bioreactor according to claim 26, wherein said at least one nozzle arrangement (10, 20) receives compressed gas (48) from a pressure vessel (44, 46).

32. The bioreactor according to claim 31, wherein said pressure vessel (44, 46) contains a bioreactive liquid substance (50) in addition to said compressed gas (48).

33. The bioreactor according to claim 32, wherein said at least one nozzle arrangement (10, 20) alternatively receives compressed air (48) or said liquid bioreactive substance (50) from said pressure vessel (44, 46).

34. The bioreactor according to claim 26, wherein said at least one nozzle arrangement (10, 20) can be pressurized with pulsing compressed air (48).

35. The bioreactor according to claim 26, wherein said second nozzle arrangement (20) is provided within said fermentation vessel (2) in a height-adjustable manner.

36. The bioreactor according to claim 32, wherein a multitude of pressure vessels (52, 54, 56, 58, 60) pressurized with compressed air (48) and connected to a mixing vessel (46) are provided which contain different liquid bioreactive substances.

37. The bioreactor according to claim 36, wherein said mixing vessel (46) has a pressure compensating means (47).

38. The bioreactor according to claim 36, wherein said pressure vessels (52, 54, 56, 58, 60) are exchangeable and can be separately autoclaved.

39. The bioreactor according to claim 30, wherein said draining channel (36) is covered by a wire mesh (38).

40. The bioreactor according to claim 26, wherein a pressure lid (8) of said fermentation vessel (2) accommodates said first nozzle arrangement (10) whose pipes (24) extend vertically from the pressure lid (8) into the reaction space (49).

41. The bioreactor according to claim 40, wherein said vertical pipes (14) of said first nozzle arrangement (10) are provided in said pressure lid (8) to be exchangeable.

42. The bioreactor according to claim 26, wherein said fermentation vessel (2) is connected through a feed line (74) with a measuring chamber (72), which is again connected through a recirculating line (84) with said fermentation vessel (2), and that said measuring chamber (72) can be pressurized for recirculating measured media.

43. The bioreactor according to claim 26, wherein a device for heat exchange is provided, especially comprising a device

(i) in which said fermentation vessel (2) has a double wall and the thus formed cavity (51a) can be flowed through with temperature-controlled heat exchange fluids through a

connecting pipe (51b) and discharge pipe (51c); and/or

(ii) which is a horizontal pipe system (20) within said fermentation vessel (2) which can be flowed through with a temperature-controlled heat exchange fluid.

44. A method for the aerobic fermentation of solid substrates, wherein a reaction medium containing such solid substrates is thoroughly mixed by compressed gas (48) supplied to the reaction medium from above.

45. The method according to claim 44, wherein said thorough mixing is effected by a continuous stream of compressed gas or by compressed gas pulses.

46. The method according to claim 44, wherein said solid substances are selected from coal, wood and loaded soils.

47. The method according to claim 46, wherein said solid substance is coal, especially brown coal (lignite).

48. The method according to claim 47, wherein a microorganism suitable for fermentation, nutrients and/or buffers are further added to the reaction mixture.

49. The method according to claim 47, wherein said brown coal or the reaction medium containing said brown coal is tyndallized together with the bioreactor prior to fermentation or prior to the addition of said microorganism.

50. The method according to claim 47, wherein:

- (i) said brown coal has a particle size of from 1 to 10 mm;
- (ii) said microorganism is selected from molds, yeasts and white rot fungi, especially *Trichoderma atroviride*;
- (iii) the pH of the reaction medium is from 5.5 to 6.0 at the beginning of the reaction;
- (iv) the pH is maintained at from 6.5 to 7.2 during the solubilization phase;
- (v) the fermentation is performed at a temperature of from 25 °C to 30 °C; and/or
- (vi) from 1 to 25 l of compressed air per liter of fermentation broth per day is passed

項目	2017年	2016年	2015年	2014年	2013年	2012年	2011年	2010年	2009年	2008年	2007年	2006年	2005年	2004年	2003年	2002年	2001年	2000年	1999年	1998年	1997年	1996年	1995年	1994年	1993年	1992年	1991年	1990年	1989年	1988年	1987年	1986年	1985年	1984年	1983年	1982年	1981年	1980年	1979年	1978年	1977年	1976年	1975年	1974年	1973年	1972年	1971年	1970年	1969年	1968年	1967年	1966年	1965年	1964年	1963年	1962年	1961年	1960年	1959年	1958年	1957年	1956年	1955年	1954年	1953年	1952年	1951年	1950年	1949年	1948年	1947年	1946年	1945年	1944年	1943年	1942年	1941年	1940年	1939年	1938年	1937年	1936年	1935年	1934年	1933年	1932年	1931年	1930年	1929年	1928年	1927年	1926年	1925年	1924年	1923年	1922年	1921年	1920年	1919年	1918年	1917年	1916年	1915年	1914年	1913年	1912年	1911年	1910年	1909年	1908年	1907年	1906年	1905年	1904年	1903年	1902年	1901年	1900年	1899年	1898年	1897年	1896年	1895年	1894年	1893年	1892年	1891年	1890年	1889年	1888年	1887年	1886年	1885年	1884年	1883年	1882年	1881年	1880年	1879年	1878年	1877年	1876年	1875年	1874年	1873年	1872年	1871年	1870年	1869年	1868年	1867年	1866年	1865年	1864年	1863年	1862年	1861年	1860年	1859年	1858年	1857年	1856年	1855年	1854年	1853年	1852年	1851年	1850年	1849年	1848年	1847年	1846年	1845年	1844年	1843年	1842年	1841年	1840年	1839年	1838年	1837年	1836年	1835年	1834年	1833年	1832年	1831年	1830年	1829年	1828年	1827年	1826年	1825年	1824年	1823年	1822年	1821年	1820年	1819年	1818年	1817年	1816年	1815年	1814年	1813年	1812年	1811年	1810年	1809年	1808年	1807年	1806年	1805年	1804年	1803年	1802年	1801年	1800年	1799年	1798年	1797年	1796年	1795年	1794年	1793年	1792年	1791年	1790年	1789年	1788年	1787年	1786年	1785年	1784年	1783年	1782年	1781年	1780年	1779年	1778年	1777年	1776年	1775年	1774年	1773年	1772年	1771年	1770年	1769年	1768年	1767年	1766年	1765年	1764年	1763年	1762年	1761年	1760年	1759年	1758年	1757年	1756年	1755年	1754年	1753年	1752年	1751年	1750年	1749年	1748年	1747年	1746年	1745年	1744年	1743年	1742年	1741年	1740年	1739年	1738年	1737年	1736年	1735年	1734年	1733年	1732年	1731年	1730年	1729年	1728年	1727年	1726年	1725年	1724年	1723年	1722年	1721年	1720年	1719年	1718年	1717年	1716年	1715年	1714年	1713年	1712年	1711年	1710年	1709年	1708年	1707年	1706年	1705年	1704年	1703年	1702年	1701年	1700年	1699年	1698年	1697年	1696年	1695年	1694年	1693年	1692年	1691年	1690年	1689年	1688年	1687年	1686年	1685年	1684年	1683年	1682年	1681年	1680年	1679年	1678年	1677年	1676年	1675年	1674年	1673年	1672年	1671年	1670年	1669年	1668年	1667年	1666年	1665年	1664年	1663年	1662年	1661年	1660年	1659年	1658年	1657年	1656年	1655年	1654年	1653年	1652年	1651年	1650年	1649年	1648年	1647年
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REMARKS

Early and favorable action is earnestly solicited.

By

KGB:ja
220 East 42nd Street
30th Floor
New York, New York 10017
Tel.: (212) 808-0700
Fax: (212) 808-0844

7/pts

SMB

Bioreactor for Fermenting Solids

The present invention relates to a bioreactor for fermenting solids, and a corresponding fermentation method.

The conversion of solid, water-insoluble or particular substances in fermenters involves a wide variety of problems, primarily relating to aeration, mixing and the addition of nutrient media. In addition, if the substrate to be converted is to be synergistically attacked by a number of different microorganisms, it is required to selectively supply the reaction space with nutrients and oxygen. In large-volume reactors, such fermentations currently cannot be realized due to the complicated mixing and the resulting defective aeration and deficient supply of substrates.

In commercially available reactor systems, the mixing is effected by mechanical agitating systems. In addition, U.S. Patent 4,846,964 describes a fluidized-bed bioreactor system for converting coal to microbiologically liquefied coal products in which an upflowing aqueous stream keeps the coal particles fluidized. The above mixing methods have a drawback in that sufficient mixing and thus a high substrate turnover is no longer possible in the fermentation of higher substrate concentrations or substrates which tend to agglomerate.

Now, it has been the object of the present invention to provide a bioreactor and a fermentation method which overcome the drawbacks of the prior art and, in particular, ensure a sufficient mixing of the substrate to be fermented.

Surprisingly, it has now been found that a sufficient mixing of the reaction medium, which contains solid or water-insoluble fermentation substrates, can be

- 2 -

achieved in a bioreactor by purposefully introducing a compressed gas continuously or in compressed gas pulses.

The above object is achieved by a bioreactor having the features of claim 1, and a method for aerobic fermentation having the features of claim 19.

The bioreactor serves for the fermentation of water-insoluble or particular substrates, such as wood (which can be degraded only by particular microorganisms due to its lignin content), coals (with the goal of using the liquid fermentation products as starting materials for the chemical industry or for thermal utilization), for the remediation of soils loaded with xenobiotics, for the rapid fermentation of organic waste products, for biological waste water purification, and for the pretreatment of basic materials of the chemical industry.

The proposed bioreactor for the first time permits the optimum aeration and mixing of solid, water-insoluble and particular substances, which is necessary for microorganisms, by using at least one specific nozzle arrangement which is introduced into the substrate to be fermented and pneumatically supplied with compressed gas. Both the supply of oxygen to the microorganisms and the supply thereto of nutrient media, cosubstrates, vitamins, minerals, buffers or antibiotics are effected by a single pneumatic pressure system. In a slightly modified form, the bioreactor can be used in any size from a five-liter laboratory scale to an industrial, multi-hectoliter scale. With simple modifications, the proposed bioreactor can also be used as a conventional liquid/solid phase, solid phase, falling film, fed batch or air-lift reactor.

According to the invention, a first, vertically extending nozzle arrangement can be extended into and retracted from the reaction space of the fermentation vessel. Thus, the nozzle arrangement can be shifted in a vertical direction to enable movement of the nozzle arrangement while pressurized with compressed gas, for a better mixing and aeration. However, if the fermentation process is to proceed under sterile conditions, it is imperative that the reaction space be kept closed. The nozzle arrangement consists of pipes which vertically protrude into the fermentation vessel and are provided with nozzles on their lower ends. In this way, the

- 3 -

compressed gas or the liquid bioreactive substrate can be introduced near the bottom of the fermentation vessel. The nozzle arrangement is also suitable for penetrating granular solids present in the fermentation broth. The vertical pipes can have different lengths and can be exchanged. Further, the nozzle arrangement, when retracted from the fermentation vessel, can be cleaned in a simple way.

In addition, a second horizontal nozzle arrangement can be provided which consists of interconnected pipes horizontally extending in parallel through the reaction space. The pipes have nozzles distributed on their coat surface.

The horizontal nozzle arrangement can be used for mixing additionally to the vertical nozzle arrangement.

If the horizontal nozzle arrangement is to rotate for a better mixing of the fermentation substrate, the vertical arrangement of nozzles must be moved upwards, or the vertical pipes must be selected so as not to hinder the rotating of the horizontal nozzle arrangement, i.e., the "normal" pipes must be replaced by shorter pipes.

An advantageous further development of the bioreactor according to the invention has a measuring device in which several measuring electrodes, for example, are provided in a measuring chamber for measuring a medium removed from the fermentation vessel. The measuring chamber is connected with the fermentation vessel through a feed line in order to feed medium to be measured from the fermentation vessel into the measuring chamber. To recirculate the medium into the fermentation vessel after measuring, the measuring chamber is further connected with the fermentation vessel through a recirculating line. The special about this arrangement is the fact that a pressure chamber is inserted upstream from the measuring chamber, where a defined pressure can build which corresponds to the displaced volume in the measuring chamber. According to the invention, the recirculation of the measured medium is effected by applying pressure to the measuring chamber so that the medium is pressed back into the fermentation vessel.

- 4 -

In known bioreactors, measurements are usually performed directly within the fermentation vessel. Since solid substances are contained in the fermentation vessel, the measuring electrodes are often damaged. For external measurement, in known bioreactors, the medium to be measured is transported into a measuring chamber by peristaltic pumps. Such pumps are subject to high wear and are not suitable for transporting larger volumes.

Because the medium is transported by pressure according to the invention, such pumps which are subject to wear and thus to intensive maintenance can be dispensed with. Further, it is possible to transport large volumes. Another advantage of transport by pressure is the fact that the sterility is not affected. This means that no foreign matter gets into the fermentation vessel during the pumping process.

The feeding of medium through the feed line into the measuring chamber can be effected by negative pressure. The measuring chamber according to the invention can further be used as an additional recirculation system. Such a recirculation system can perform an additional aeration of the substrates present in the fermentation vessel.

Further advantageous embodiments of the bioreactor can be seen from the further dependent claims.

In the method according to the invention, for the aerobic fermentation of solid substrates, a reaction medium containing such solid substrates is thoroughly mixed by compressed gas introduced from above into the reaction medium. This thorough mixing dramatically increases the fermentation rate. According to the present invention, "to introduce from above into the reaction medium" means that compressed gas is introduced into the reaction medium by means of suitable devices (such as pipes provided with nozzles) which extend into the reaction medium from above. The length of the pipes can be selected to reach below the surface of the reaction medium at any level in the introduced working condition (i.e., with or without introduced compressed gas).

- 5 -

In the following, the invention will be further described by the Figures and Examples, wherein:

Figure 1 shows a bioreactor having a pressure vessel for bioreactive substances and for compressed air;

Figure 2 shows a frontal view of the bioreactor;

Figure 3 shows a side view of the bioreactor;

Figure 4 shows a cross-sectional view through the fermentation vessel of the bioreactor;

Figure 5 shows a cross-sectional view along line V-V in Figure 4;

Figure 6 shows a media mixing and compressed air feeding arrangement for the bioreactor;

Figure 7 is a schematic view of a measuring means connected with the fermentation vessel; and

Figure 8 shows the course of reaction of the fermentation charge described in Example 1.

The bioreactor shown in Figure 1 for the fermentation of solid substrates and for performing one of the fermentation processes described in more detail below has a fermentation vessel 2 which can be hermetically sealed with a pressure lid 8.

Fermentation vessel 2 is provided with at least one nozzle arrangement 10, 20 extending into the reactor space 49 to which compressed air 48 is supplied through a pressure vessel 44, through a compressed gas line 3 for a vertical nozzle arrangement 10 and through a compressed gas line 4 for a horizontal nozzle arrangement 20.

- 6 -

The pressure vessel 44 additionally contains a bioreactive liquid substance 50 which can be supplied through a stop valve 7 and a pressure line 5 to the horizontal nozzle arrangement 20, and/or through a pressure line 9 to the vertical nozzle arrangement 10.

Consequently, the vertical nozzle arrangement 10 and/or the horizontal nozzle arrangement 20 may also be used as a supply means for bioreactive substances as an alternative of pressurizing with compressed gas.

On the bottom of the pressure vessel 44 is provided a discharge line 11 with a stop valve 12. On the top end of the pressure vessel 44 is provided a feed line 13 for supplying bioreactive substances 50 and a stop valve 15.

The compressed gas 48, e.g., compressed air, is supplied through a feed line 17 and a stop valve 18.

Juxtaposed to the pressure vessel 44 is a rising pipe 19.

The fermentation 2 further has a pressure compensating means 21 with a stop valve. On the bottom of the fermentation vessel 2, a draining channel 36 covered by a wire mesh 38 is provided below a conical bottom section 32 (Figure 2), so that liquid contained in the fermentation vessel 2 is coarse-filtered prior to being discharged into the draining channel. The bottom of the draining channel 36 is inclined from horizontal, a draining valve 40 being provided at the front side 51 of the fermentation vessel at the lowest position of the draining channel 36.

On the front side 51 of the fermentation vessel 2 is provided a removable door 53 to enable cleaning of the interior space and of the wire mesh 38 without having to open the cover plate 8 sealing the reactor space 49. On the draining channel 36 are provided two windows 41 facing each other, which enable determination of the optical density of the fermented substance with an optical sensor (Figure 3). Additional windows 62, 64 provided on the lateral walls enable visual control of the fermentation conditions, but these are not obligatory.

- 7 -

Instead of or in addition to a subsequently described external measuring chamber 72, on one side of the fermentation vessel 2, there may be provided a sealable means 66 for measuring electrodes (e.g., pH, O₂ partial pressure, conductivity, ion specificity etc.) and a pressure compensating means 21 for the controlled exhausting of air or gases formed during fermentation from the fermentation vessel 2.

All components which will come into contact with medium or gases can be independently autoclaved.

The bioreactor according to the invention can further have devices for heat exchange, i.e., heating and cooling. Thus, the fermentation vessel 2 can have a double wall to form a cavity 51a. This cavity is formed between the inner and outer walls of vessel 2. The cavity 51a is connected, for example, with a correspondingly temperature-controlled heat exchange fluid reservoir (e.g., water or oil reservoir) through a connecting pipe 51b and discharge pipe 51c. The water or oil which has a defined temperature due to an external thermostat flows around vessel 2. Due to this device, the bioreactor can be operated with stepless control in a temperature range of from 10 to 90 °C, whereby, on the one hand, the optimum temperature for the different microorganisms can be adjusted in the bioreactor, and on the other hand, the whole bioreactor including its contents can be mildly sterilized or tyndallized. Therefore, such a sterilization/tyndallization of a whole bioreactor in an integrated system, which has not been previously described as such, has significant advantages in the application of the system.

As can be seen from Figures 2 and 3, the fermentation vessel 2 is held by a frame 6. The frame 6 also accommodates two spindles 22 by means of which the pressure lid 8 of fermentation vessel 2 can be lowered onto the latter or lifted. The pressure lid 8 at the same time forms the vertical nozzle arrangement by effecting the supply of compressed gas or bioreactive substances through the pressure lid 8 to the vertical pipes 14 extending vertically from the pressure lid 8. The vertical pipes 14 can be of different lengths, the tips of the pipes 14 with nozzles 16 being positioned in the bottom portion of the fermentation vessel when the pressure lid 8 is closed. By lowering the pressure lid, the nozzles 16 can also penetrate solid

- 8 -

substrates present in the reactor space. In the closed position of the pressure lid, the fermentation vessel 2 can be hermetically sealed.

The spindles 22 are actuated by means of crank handles 27 provided on the top of frame 6.

A hollow pressure plate 8 serves as the pressure lid of bioreactor 1, being one of two possibilities for aerating the bioreactor space in the fermentation vessel 2 or supplying it with media. It can be vertically guided by rails and hermetically seals the fermentation vessel 2 during operation. When the bioreactor 1 is to be charged with the substrate to be converted, the pressure lid 8 can also be lifted hydraulically or pneumatically in addition to the mechanical way described above.

On its top side, pressure lid 8 has a manually or electronically controllable port for supplying compressed air (or defined gases). Optionally, the port may also be used for the supply of liquid defined media, e.g., required cosubstrates, vitamins, minerals, buffers or antibiotics. Within pressure lid 8, the air first impinges on a baffle 45. On the lower side of pressure lid 8, exchangeable pipes 14 with nozzles 16 are screwed into threads. When pressure builds in the pressure lid 8, the air continuously flows through the nozzles 16 provided at the tips of pipes 14 into the reactor space 49 of fermentation vessel 2, aerating it uniformly. In addition to the mild mixing of the reactor space by the continuous flow of air, a thorough mixing of the reactor space can optionally be effected by strong pulses of compressed air applied in defined intervals.

Due to the different lengths of the exchangeable pipes 14 which can be screwed into the pressure lid 8, it is possible to aerate the reactor space 49 in a well-aimed manner. By nozzles 16 extending to the bottom of fermentation vessel 2, a homogeneous aerobic reaction space can be achieved. When shorter nozzles are selected, a defined space having a lower oxygen partial pressure is generated. The bioreactor 1 can be operated either without gassing (in which case the threads of pressure lid 8 can be closed by blind stoppers), or in an obligatory anaerobic mode by supplying defined gases free of O_2 . In this case, any media to be added should be degassed before being supplied.

Optionally, liquid media may also be added to bioreactor 1 from the pressure or mixing vessel 44, 46 through these nozzles 16. In this case, aeration, if required, may also be effected by the nozzle arrangement 20 extending horizontally through reactor space 49.

Figures 4 and 5 show the nozzle arrangement 20 provided horizontally within fermentation vessel 2, which can be pressurized with either compressed air 48 or a liquid bioreactive substance 50. As can be seen from Figure 5, the horizontal nozzle arrangement 20 can be supported on one side in a front wall of fermentation vessel 2 or on both sides in the front walls. Further, the horizontal nozzle arrangement may be designated for support at different levels on the front wall.

As can be seen from Figure 5, the horizontal nozzle arrangement 20 consists of three pipes 24 each having a plurality of nozzle orifices 28 extend horizontally in parallel through fermentation vessel 2. The three pipes 24 are interconnected through an inlet manifold 20a and an outlet manifold 20b.

As can be seen from Figure 5, the horizontal nozzle arrangement 20 can be rotated around a horizontal rotation axis to additionally achieve a thorough mixing of the vessel contents. When the horizontal nozzle arrangement 20 is rotated, the length of pipes 14 of the vertical nozzle arrangement 10 is to be selected to preclude collision between pipes 14 and pipes 24. Alternatively, if sterility of the fermentation process is not required, the vertical nozzle arrangement may be retracted vertically upwards to such an extent that a collision between pipes 14 and 24 cannot occur. To maintain sterile conditions, the rotating horizontal nozzle arrangement 20 is preferably provided at the lowest possible position within fermentation vessel 2. In this case, the pipes 14 are preferably shortened to the extent as just not extending into the rotating area. In this case, the outermost pipes 14 which are provided close to the sides of fermentation vessel 2 are longer than the pipes provided further inwardly.

The outlet manifold 20b can have a valve for closing the outlet, or it may also be used as an inlet manifold, in which case the material supplied to the pipes 24 must completely pass through the nozzle orifices 28. The outlet manifold 20b may also

The nozzle arrangement 20 may also be used for heating/cooling by closing the nozzles 28, or by replacing the nozzle arrangement 20 by a corresponding pipe system without nozzles. In this case, the inlet manifold 20a serves for supplying the temperature-controlled heating/cooling fluid, and the outlet manifold 20b serves as a discharge thereof. Such a nozzle arrangement 20 designed as a cooling arrangement may also be rotating. Further, several nozzle/cooling arrangements may be provided, so that a rotating horizontal nozzle arrangement and a rotating horizontal cooling arrangement, for example, can be provided simultaneously in one vessel 2.

Of course, it is possible to pressurize the vertical nozzle arrangement 10 with compressed gas 48 and to pressurize the horizontal nozzle arrangement 20 with the bioreactive substance 50, or vice versa.

The second nozzle arrangement 20 extends horizontally through the reactor space 49. It can be introduced at different levels and is supported to rotate around its axis. Such a nozzle arrangement is used for supplying the bioreactor with liquid defined media, e.g., water, required cosubstrates, vitamins, minerals, buffers or antibiotics, but may optionally be used for aerating and mixing the bioreactor 1. For the fermentation of liquid or water-soluble substances, the horizontal nozzle arrangement may be rotated by a motor provided on the outer side of bioreactor 1 and thus contribute to a thorough mixing of the bioreactor contents. In this case, shorter vertical pipes 14 are employed, and the horizontal nozzle arrangement 20 is inserted in its lowermost lock.

The mixing of the bioreactor contents in the fermentation of solid substances and the supply of the bioreactor 1 with both atmospheric oxygen or defined gases and medium is achieved pneumatically by compressed air. Via a pressure vessel 44, which in this case is also used as a media storage tank, the air gets into the pressure lid 8 and from there into the vertical or optionally into the horizontal nozzles 16, 28. Between the pressure vessel 44 and the pressure lid 8, there is a

stop valve 26. When the stop valve 26 is closed and the stop valve 7 on the bottom of pressure vessel 44 is opened, medium is pressed by the compressed air through the horizontal and optionally also through the vertical nozzle arrangement 10, 20 into bioreactor 1.

Figure 6 shows an embodiment in which a mixing vessel 46 is connected with the bioreactor 1 instead of pressure vessel 44. Connected to mixing vessel 46 are several pressure vessels 52, 54, 56, 58, 60 which contain different liquid bioreactive substances as well as compressed gas, e.g., compressed air. These different substances can be supplied to the mixing vessel 46, where they can be mixed at a desired ratio. Through the compressed air feed line 17 and the stop valve 18, compressed air gets to a distributing means 23 which distributes the compressed air 48 to all connected pressure vessels 52, 54, 56, 58 and 60, and to the mixing vessel 46. The compressed air feed lines 25 for the individual pressure vessels each have one stop valve 26. The compressed air line 3 for the fermentation vessel 2 also branches off from the distributing means 23.

To be able to selectively introduce different media into the presented bioreactor 1, the pneumatic system is used from several pressure vessels 52, 54, 56, 58, 60 having different volumes. Figure 6 shows an application with five exchangeable and separately autoclavable pressure vessels (e.g., for medium, two buffers, trace element solution, and antibiotics solution) in which graduated rising pipes 19 indicate the respective filling levels of pressure vessels 52, 54, 56, 58, 60. Through a distributing means 23, the compressed air gets into the pneumatic system. If needed, a part thereof can be directly introduced into the vertical and/or horizontal nozzle arrangement 10, 20 for aerating the bioreactor 1. The independently controllable pressure vessels are connected with the distributing means 23. The respective media from the individual pressure vessels are added to a mixing vessel 46. When the mixing vessel is filled with the different media, a pressure compensation is enabled by a pressure compensating means 47, and compressed air, for example, is pressed into the bottom of the pressure vessel through another compressed gas line 34 of the distributing means 23. This effects the mixing of the different solutions. When the mixing process is completed, the mixing vessel 46 is

- 12 -

pressurized with compressed air, and the desired medium is introduced into the horizontal and/or vertical nozzle arrangement 10, 20.

Each pressure vessel may be provided with a rising pipe 19. The feed lines 29 for the bioreactive substances from the pressure vessels each have a stop valve 30.

From the distributing means 23, a compressed gas line 34 further branches off which contains a stop valve 35, wherein the compressed gas line 34 leads to a discharge line 37 at the bottom of the mixing vessel 46 in order to supply compressed air, for example, for the mixing process. The discharge line is provided with a stop valve 39. From the discharge line 37, a feed line 42 with a stop valve 43 branches through which the mixed bioreactive substances 50 can be supplied to the bioreactor 1.

The mixing vessel 46 is further provided with a pressure compensating means 47 for compensating the pressure during the mixing process.

The compressed gas may also be supplied in a pulsing manner to the vertical and/or horizontal nozzle arrangement 10, 20.

A measuring means 70 is connected with the fermentation vessel 2. This measuring means 70 has a measuring chamber 72 which is connected with the fermentation vessel 2 through a feed line 74. Through the feed line 74, the medium to be measured flows from the fermentation vessel 2 into the measuring chamber 72. To set the direction of flow from the fermentation vessel 2 into the measuring chamber 72 independently of the conditions in the measuring chamber 72, a check valve 76 is provided in the feed line 74.

In the measuring chamber 72, several measuring electrodes 78 are provided which are connected through lines 80 with a measuring means 82, which is preferably computer-controlled. The measuring electrodes 78 can effect measurement of, for example, pH, oxygen, temperature and ions.

- 13 -

At the lowest position of the essentially diamond-shaped measuring chamber 72, a recirculating line 84 is connected with the measuring chamber 72. Through the recirculating line 84, the measured medium is recirculated into the fermentation vessel 2.

To recirculate the medium into the fermentation vessel 2, pressure is applied to the measuring chamber 72. For this purpose, a pressure chamber 86 is assigned to the measuring chamber 72. The pressure chamber 86 is connected with a source of compressed air through lines 88, 90. To produce a positive pressure in the pressure chamber 86, a valve 92 is opened, and a valve 94 is closed, so that the compressed air flows in the direction of arrows 96 into the pressure chamber 86.

To recirculate medium from the measuring chamber 72 through the recirculating line 84 into the fermentation vessel 2, valve 92 is closed, and valve 94 is opened. Thus, compressed air flows from the pressure chamber 86 in the direction of arrows 98 through a line 100 connected with the measuring chamber 72 into measuring chamber 72 and there produces a positive pressure. The defined positive pressure in pressure chamber 86 is directly proportional to the volume of liquid to be displaced in measuring chamber 72. Due to the positive pressure produced in measuring chamber 72, the check valve 76 in the feed line 74 is closed, and the medium present in the measuring chamber 72 is pressed back through recirculating line 84 into the fermentation vessel 2. To achieve a uniform distribution of the air pressure within the measuring chamber and to avoid eddies, a baffle 102 is provided in the region of the inlet of compressed air into measuring chamber 72.

Once the medium has been recirculated from the measuring chamber 72 into the fermentation vessel 2, valve 94 is again closed, and valve 92 is opened. Thereby, a positive pressure again builds within pressure chamber 86. Once the valve 94 is closed, a compensation of pressure occurs in the measuring chamber 72, so that the check valve 76 is again opened by medium flowing from the fermentation vessel 2. The valve 76 may further be a switching valve, for example, in order to control the quantity of medium to be measured which is supplied to the measuring chamber 72.

The present invention also relates to a method for the aerobic fermentation of solid substances, wherein the reaction medium containing said solid substances is mixed by compressed gas 48 supplied to the reaction medium from above. According to the invention, this is effected by introducing the compressed gas directly into the reaction medium using suitable means (such as a vertical nozzle arrangement 10 as described above), so that the spreading gas bubbles effect mixing. Since compressed gas rich in oxygen (such as air, O₂-enriched air or O₂) is preferably used in aerobic fermentation, the oxygen content in the reaction medium is also increased by this method, which usually accelerates the fermentation additionally. The introducing of the compressed gas may be effected continuously (also referred to as "aeration" in the following) or by pulses of compressed gas (also referred to as "thorough mixing" in the following).

"Solid substrates" within the meaning of the method according to the invention are preferably coal, wood and loaded soils. The method according to the invention is particularly suitable for fermenting coal, especially brown coal. The latter consists of three components defined by their different solubilities as a function of pH:

1. humic acids which can be extracted by 0.1 N NaOH solution;
2. fulvic acids which are also soluble in an acidic medium;
3. the insoluble residue, referred to as the "matrix".

For liquefying the brown coal, the starting product, which may optionally be pretreated or pre-oxidized, in a milled condition (particle size preferably from 0.1 mm to 2 cm, more preferably from 1 to 10 mm), is mixed with an amount of solvent (i.e., water or aqueous-based solvent systems), nutrients, buffers (including buffer substances, acids, bases) and microorganism culture sufficient for solubilization, and cultured with thorough mixing with oxygen-containing compressed gas.

Suitable microorganisms for the solubilization of brown coal include molds, white rot fungi and yeasts. One preferred microorganism for this application is *Trichoderma atroviride*. The nutrients to be employed for this fermentation method highly depend on the species of microorganism employed. In particular, it is

preferred to add carbon sources to the microorganism which liquefies the brown coal at the beginning of the reaction to ensure a growth advantage.

Buffer substances which ensure the desired pH value at the respective times of reaction are employed. Thus, at the beginning of the reaction, when a pH of from 5.5 to 6.0 is preferred, and in the liquefying phase, when a pH of from 6.5 to 7.2 is preferred, a citrate/phosphate buffer having a pH of 3 is preferably used, since the fungus itself alkalizes the medium when growing, and only back titration must be performed. It is particularly preferred to set a pH of 5.5 at the beginning of the experiment. The fermentation is preferably performed at a temperature of from 15 to 35 °C.

In a preferred embodiment, the brown coal, the brown coal/solvent mixture or the brown coal/solvent/nutrient mixture is sterilized or tyndallized prior to the addition of the fermenting microorganisms. This is preferably performed by several cycles of heating at temperatures of above 75 °C, preferably above 80 °C, for at least 45 minutes, followed by cooling down to room temperature for several hours.

In the 80 °C steps, the physiologically active microorganisms in the substrate, medium and reactor space, but not dormant spores, are killed. In the periods of time with moderate temperatures, the spores germinate, and are killed in the subsequent heating step.

Conventional tyndallization means three cycles of heating of a liquid or nutrient medium. In the intervals between the temperature steps, the product is kept at room temperature (Eckhard Bast, 1999, Mikrobiologische Methoden: eine Einführung in grundlegende Arbeitstechniken - Heidelberg, Berlin; Spektrum Akademischer Verlag, ISBN 3-8274-0786-9).

The microbially solubilized coal obtainable according to the present invention can be used as a carbon and energy source for bacteria which are capable of producing a chemically characterized substance, such as polyhydroxyfatty acids for the synthesis of biodegradable plastics, from the chemically heterogeneous mass product coal (A. Steinbüchel and B. Fuchtenbusch, Proceedings ICCS 97, 1673-

1676 (1997)). The aliphatic residue which cannot be liquefied microbially and has a lower proportion of water and ashes and thus a higher gross calorific value can be used, on the one hand, for direct thermal utilization (R. Köpsel et al., Freiburger Forschungshefte, 159-166 (1998)), and on the other hand, for further subsequent fermentation processes by aliphatic-degrading yeasts (U. Hölker et al., Proceedings of the 16th SMYTE, Slovakia, page 16 (1998); Folia Microbiol. 44, 226-227 (1999)).

It is particularly preferred to perform the method according to the invention in the above described bioreactor.

The present invention is further illustrated by the following Example.

Examples

General procedures

The product "liquefied coal" was defined as the sample supernatant obtained after 20 min of centrifuging at 10,000 x g. The degree of liquefaction was determined from the optical density at 450 nm or from the dry weight of the supernatant. To separate the humic acid compounds from fulvic acid compounds, the supernatant was acidified to pH 1.5 and again centrifuged. The liquefied products were characterized with respect to their optical densities, humic and fulvic acid contents and bacterial contamination by means of incubation of samples in complete media followed by microscopic analyses.

Example 1: Fermentation of brown coal

A bioreactor according to the invention (as shown in Figures 1 to 6 having a vertical nozzle arrangement (10), a pressure lid (8) with pipes (14) having nozzles (16) and extending to the bottom of the reactor space (49), and a rigid horizontal nozzle arrangement (20); volume: 25 l) was charged with 2,500 g of brown coal (Bergheim Lithotyp A, particle size from 2 to 10 mm, water content of the coal about 50%) as a solid to be converted in 10 l of water. As the coal-solubilizing

A bioreactor according to the invention (as shown in Figures 1 to 6, having an insertable vertical nozzle arrangement (10) with short pipes (14), devices for heat exchange (51a, 51b, 51c) in the outer wall of a rotating horizontal nozzle ar-

To verify that a successful sterilization had taken place, the bioreactor was subsequently further thoroughly mixed, aerated and recirculated. Daily (for 7 days), 1 ml samples were taken and used to inoculate both Petri dishes (1.2% agar) and 50 ml liquid cultures containing the nutrient medium used in the bioreactor. The Petri dishes were incubated at 25 °C for 72 hours, and the liquid cultures were incubated at 25 °C and 120 rpm on a vibrator. It was found that no contaminations could be detected under such conditions in the reactor space.

After the sterility control had been performed, the bioreactor was inoculated with the coal-solubilizing fungus *T. atroviride*, and the coal was fermented by analogy with Example 1, but at a set temperature of 25 °C to obtain 60 g of liquefied product.

CLAIMS:

(amended August 24, 2001)

1. A bioreactor for fermenting solid substrates, comprising a fermentation vessel (2), a charging means for bioreactive substances, and a nozzle arrangement within said fermentation vessel (2) for aeration and thorough mixing of the substrates, wherein the at least one nozzle arrangement (10, 20) has a multitude of pipes (14, 24) extending in parallel into the reaction space (49) of the fermentation vessel (2) and provided with nozzles (16, 28), wherein a first, vertically extending nozzle arrangement (10) can be extended into and retracted from the reaction space (49) of the fermentation vessel (2), characterized by a second, horizontal nozzle arrangement (20) consisting of at least two interconnected pipes (24) each having a plurality of nozzle orifices (28) and extending horizontally and in parallel through the reaction space (49).
2. The bioreactor according to claim 1, characterized in that said horizontal nozzle arrangement (20) can be rotated around a horizontal rotation axis.
3. The bioreactor according to claim 1 or 2, characterized in that said fermentation vessel (2) has a bottom section (32) with a tapering cross-section.
4. The bioreactor according to claim 3, characterized in that said conically designed bottom section (32) leads into a draining channel (36) which is inclined from horizontal and has a draining valve (40) at the lowest position thereof.
5. The bioreactor according to one or more of claims 1 to 4, characterized in that said at least one nozzle arrangement (10, 20) receives compressed gas (48) from a pressure vessel (44, 46).
6. The bioreactor according to claim 5, characterized in that said pressure vessel (44, 46) contains a bioreactive liquid substance (50) in addition to said compressed gas (48).

- 2 -

7. The bioreactor according to claim 6, characterized in that said at least one nozzle arrangement (10, 20) alternatively receives compressed air (48) or said liquid bioreactive substance (50) from said pressure vessel (44, 46).
8. The bioreactor according to one or more of claims 1 to 7, characterized in that said at least one nozzle arrangement (10, 20) can be pressurized with pulsing compressed air (48).
9. The bioreactor according to one or more of claims 1 to 8, characterized in that said second nozzle arrangement (20) is provided within said fermentation vessel (2) in a height-adjustable manner.
10. The bioreactor according to claim 6, characterized in that a multitude of pressure vessels (52, 54, 56, 58, 60) pressurized with compressed air (48) and connected to a mixing vessel (46) are provided which contain different liquid bioreactive substances.
11. The bioreactor according to claim 10, characterized in that said mixing vessel (46) has a pressure compensating means (47).
12. The bioreactor according to claim 10 or 11, characterized in that said pressure vessels (52, 54, 56, 58, 60) are exchangeable and can be separately autoclaved.
13. The bioreactor according to one or more of claims 4 to 12, characterized in that said draining channel (36) is covered by a wire mesh (38).
14. The bioreactor according to one or more of claims 1 to 13, characterized in that a pressure lid (8) of said fermentation vessel (2) accommodates said first nozzle arrangement (10) whose pipes (24) extend vertically from the pressure lid (8) into the reaction space (49).

15. The bioreactor according to claim 14, characterized in that said vertical pipes (14) of said first nozzle arrangement (10) are provided in said pressure lid (8) to be exchangeable.
16. The bioreactor according to one or more of claims 1 to 15, characterized in that said fermentation vessel (2) is connected through a feed line (74) with a measuring chamber (72), which is again connected through a recirculating line (84) with said fermentation vessel (2), and that said measuring chamber (72) can be pressurized for recirculating measured media.
17. The bioreactor according to one or more of claims 1 to 16, characterized in that a device for heat exchange is provided, especially comprising a device
 - (i) in which said fermentation vessel (2) has a double wall and the thus formed cavity (51a) can be flowed through with temperature-controlled heat exchange fluids through a connecting pipe (51b) and discharge pipe (51c); and/or
 - (ii) which is a horizontal pipe system (20) within said fermentation vessel (2) which can be flowed through with a temperature-controlled heat exchange fluid.
18. A method for the aerobic fermentation of solid substrates, wherein a reaction medium containing such solid substrates is thoroughly mixed by compressed gas (48) supplied to the reaction medium from above.
19. The method according to claim 18, wherein said thorough mixing is effected by a continuous stream of compressed gas or by compressed gas pulses.
20. The method according to claim 18 or 19, wherein said solid substances are selected from coal, wood and loaded soils.
21. The method according to claim 20, wherein said solid substance is coal, especially brown coal (lignite).

22. The method according to claim 21, wherein a microorganism suitable for fermentation, nutrients and/or buffers are further added to the reaction mixture.
23. The method according to claim 21, wherein said brown coal or the reaction medium containing said brown coal is tyndallized together with the bioreactor prior to fermentation or prior to the addition of said microorganism.
24. The method according to one or more of claims 21 to 23, wherein:
 - (i) said brown coal has a particle size of from 1 to 10 mm;
 - (ii) said microorganism is selected from molds, yeasts and white rot fungi, especially *Trichoderma atroviride*;
 - (iii) the pH of the reaction medium is from 5.5 to 6.0 at the beginning of the reaction;
 - (iv) the pH is maintained at from 6.5 to 7.2 during the solubilization phase;
 - (v) the fermentation is performed at a temperature of from 25 °C to 30 °C; and/or
 - (vi) from 1 to 25 l of compressed air per liter of fermentation broth per day is passed through the reaction medium.
25. The method according to one or more of claims 18 to 24, wherein said reaction is performed in a bioreactor (1) as defined in claims 1 to 17.

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(72) Erfinder: HÖLKER, Udo [DE/DE]; Rotdomstr. 2,
D-53639 Königswinter-Rauschendorf (DE).

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(74) Anwälte: HELBING, Jörg usw.; Von Kreisler Selting
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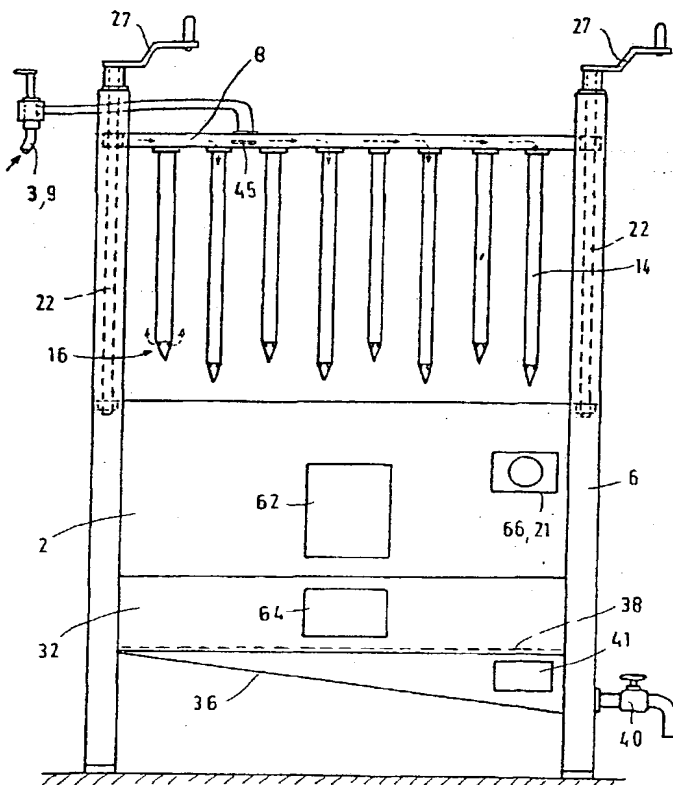
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(54) Bezeichnung: BIOREAKTOR ZUR FERMENTIERUNG VON FESTEN STOFFEN



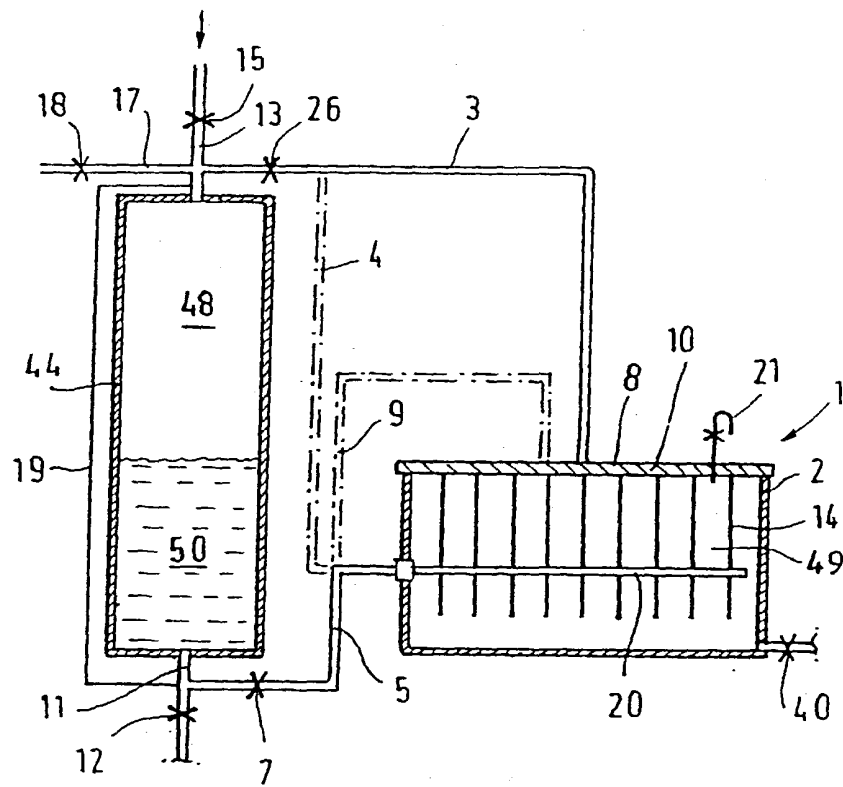
(57) Abstract: The invention relates to a bioreactor for fermenting solid substrates, comprising a fermentation vessel (2), a device for feeding bioreactive substances and a nozzle arrangement in the fermentation vessel (2). The invention is characterized in that a nozzle arrangement (10, 20) consisting of a plurality of pipes (14, 24) which project into the reaction chamber (49) of the fermentation vessel (2) in parallel and which are provided with nozzles (16, 28) is situated in the fermentation vessel (2). The invention also relates to a method for aerobically fermenting solids. A reaction medium containing these solids is mixed using a compressed gas (48) which is guided into the reaction mixture from above.

(57) Zusammenfassung: Die Erfindung betrifft einen Bioreaktor zur Fermentierung fester Substrate mit einem Fermentationsbehälter (2), einer Zugabeeinrichtung für bioreaktive Stoffe und einer Düsenanordnung in dem Fermentationsbehälter (2), dadurch gekennzeichnet, dass eine Düsenanordnung (10, 20) aus einer Vielzahl von parallel in den Reaktionsraum (49) des Fermentationsbehälters (2) hineinragenden und mit Düsen (16, 28) versehenen Leitungsrohren (14, 24) in dem Fermentationsbehälter (2) angeordnet ist. Die Erfindung betrifft weiterhin ein Verfahren zur aeroben Fermentierung fester Stoffe, wobei ein diese festen Stoffe enthaltendes Reaktionsmedium durch von oben in das Reaktionsgemisch zugeführtes Druckgas (48) durchmischt wird.

WO 01/19954 A1

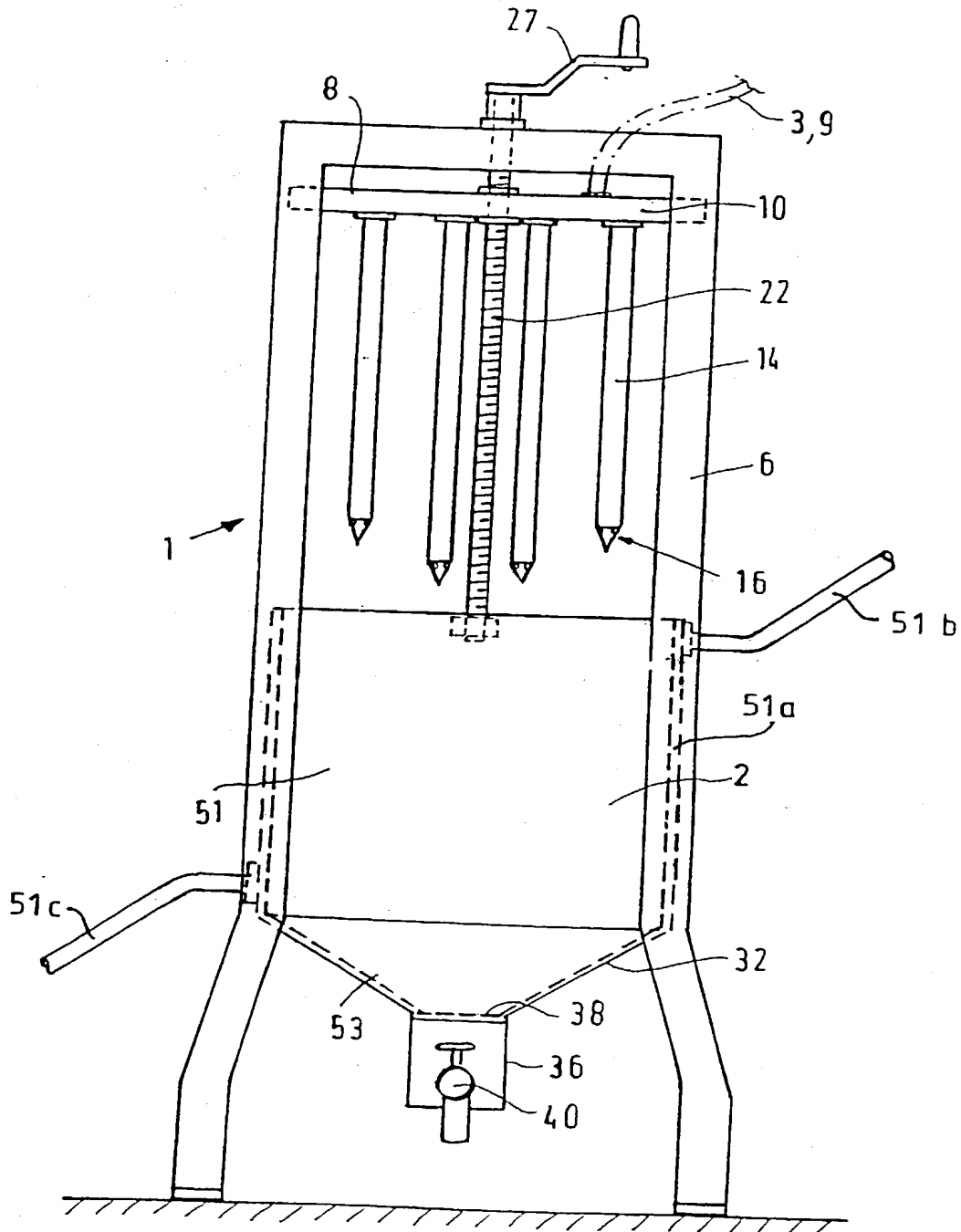
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FIG.1



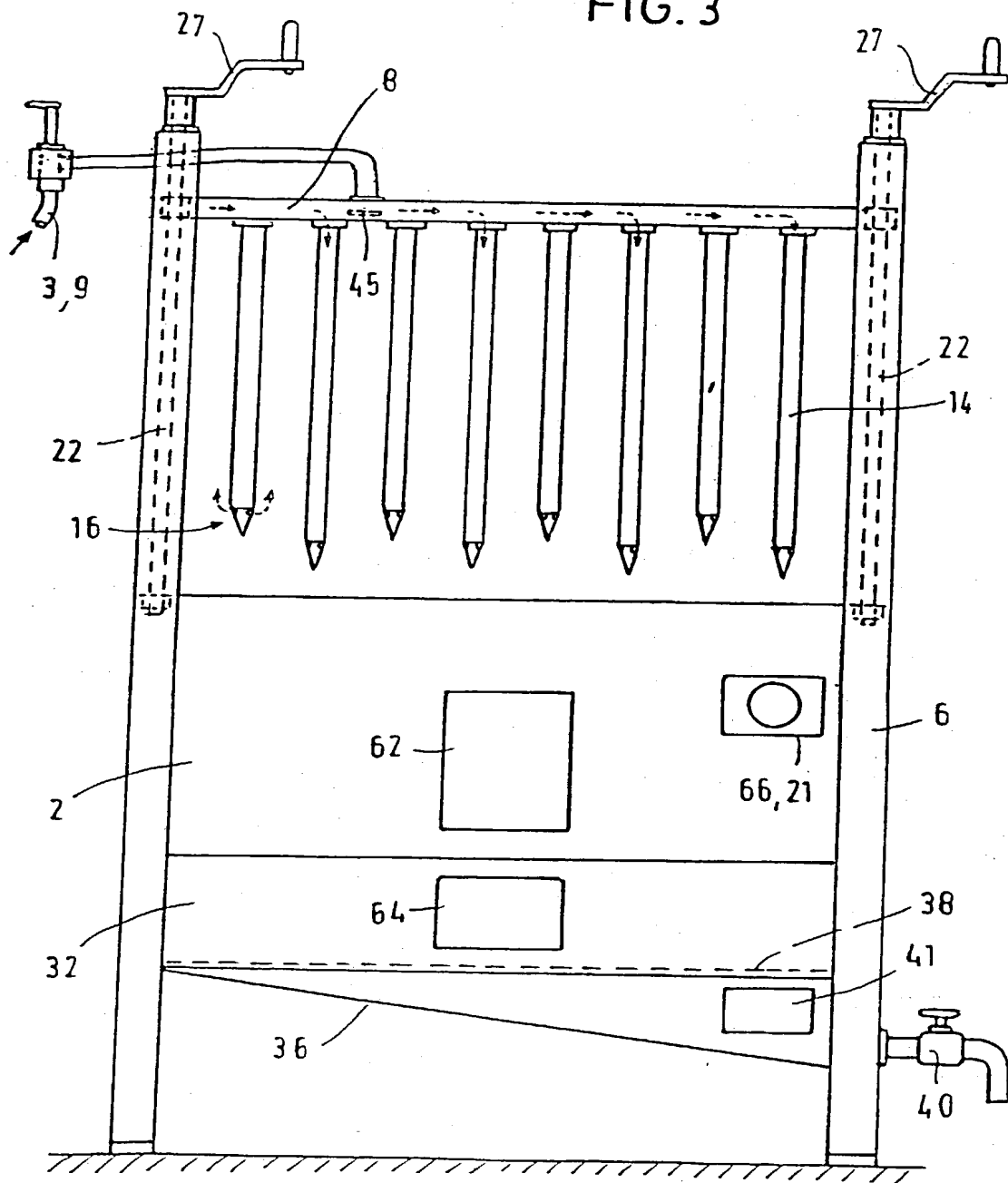
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FIG.2



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FIG. 3



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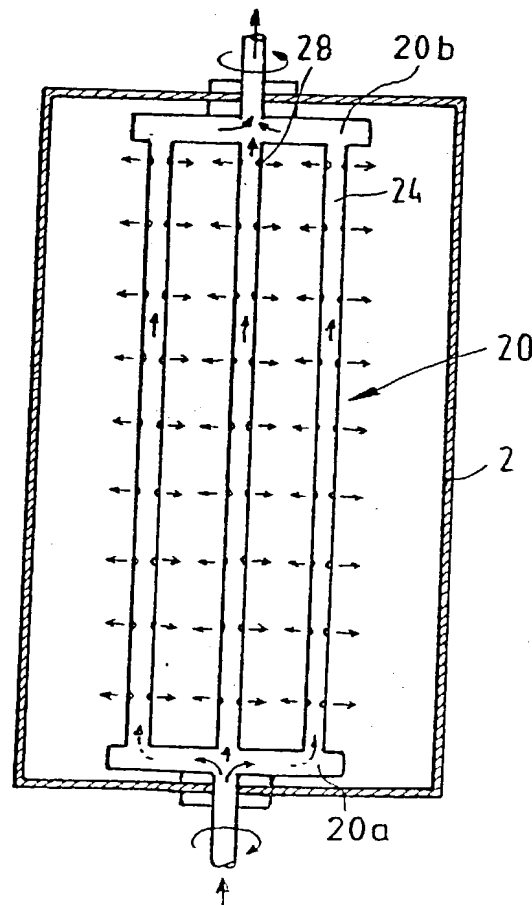
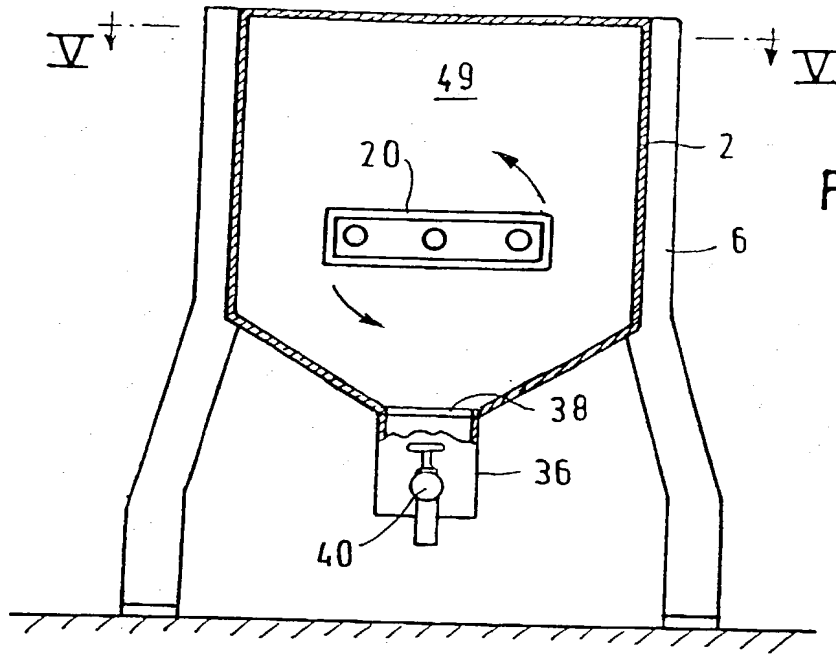
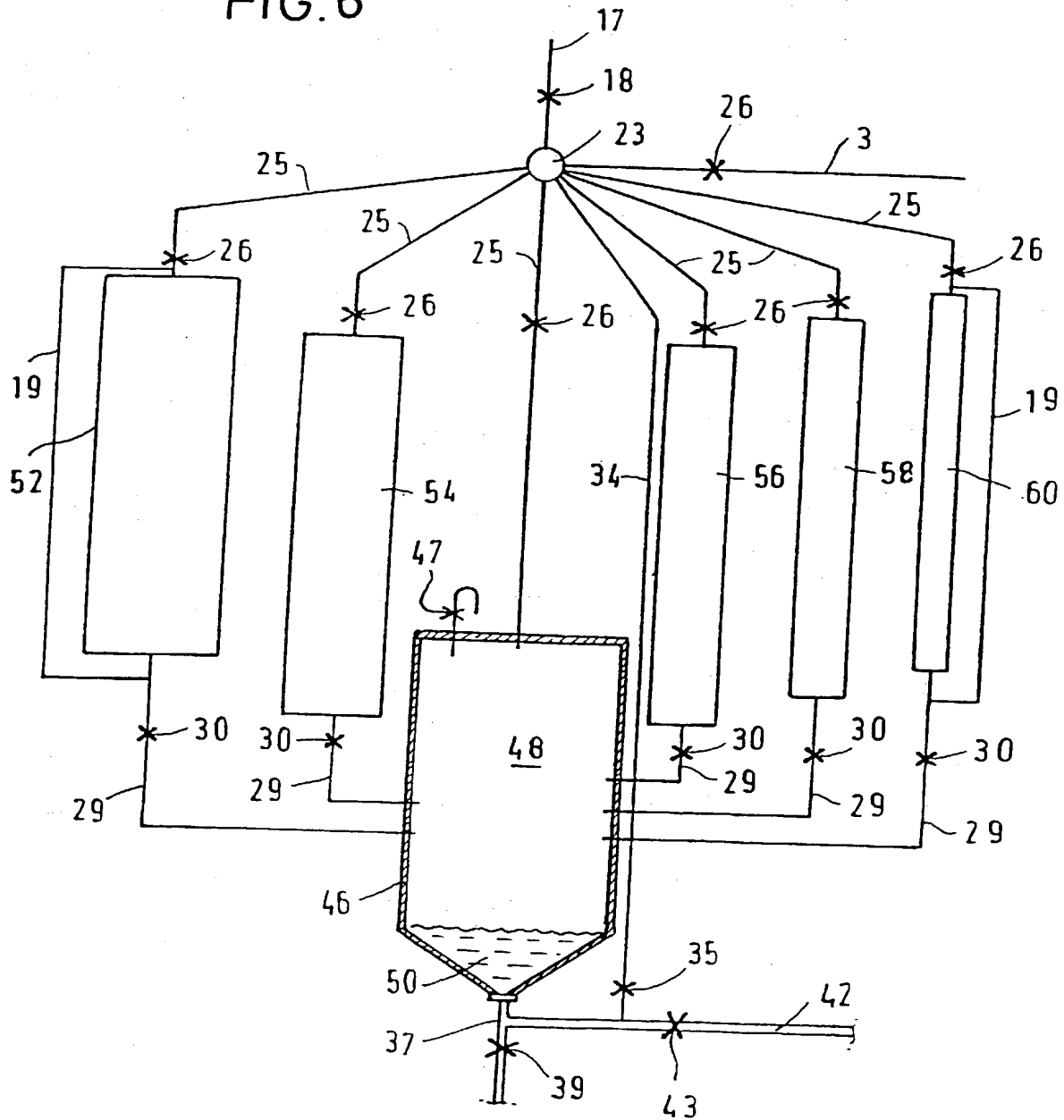


FIG. 6



- 6/7 -

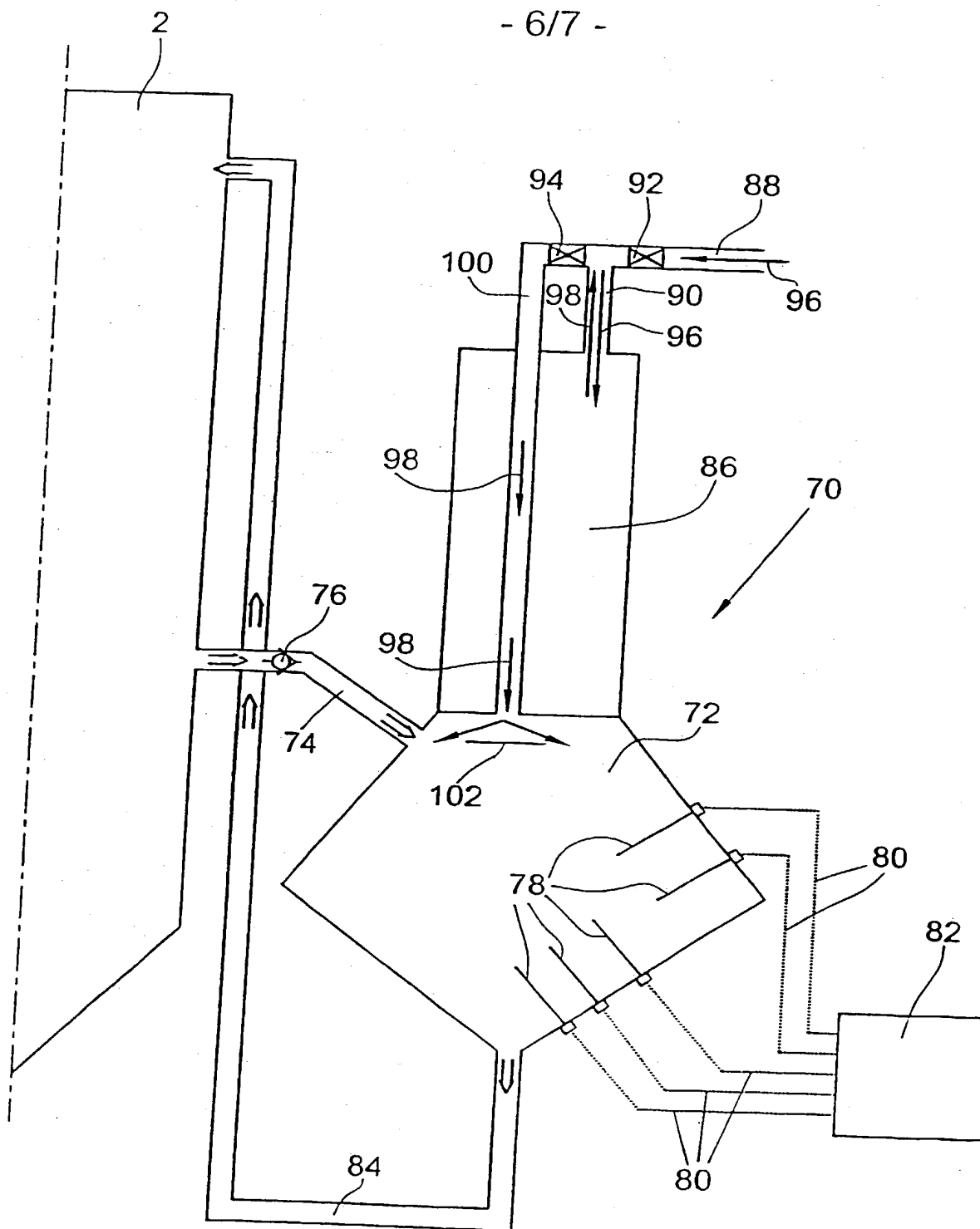
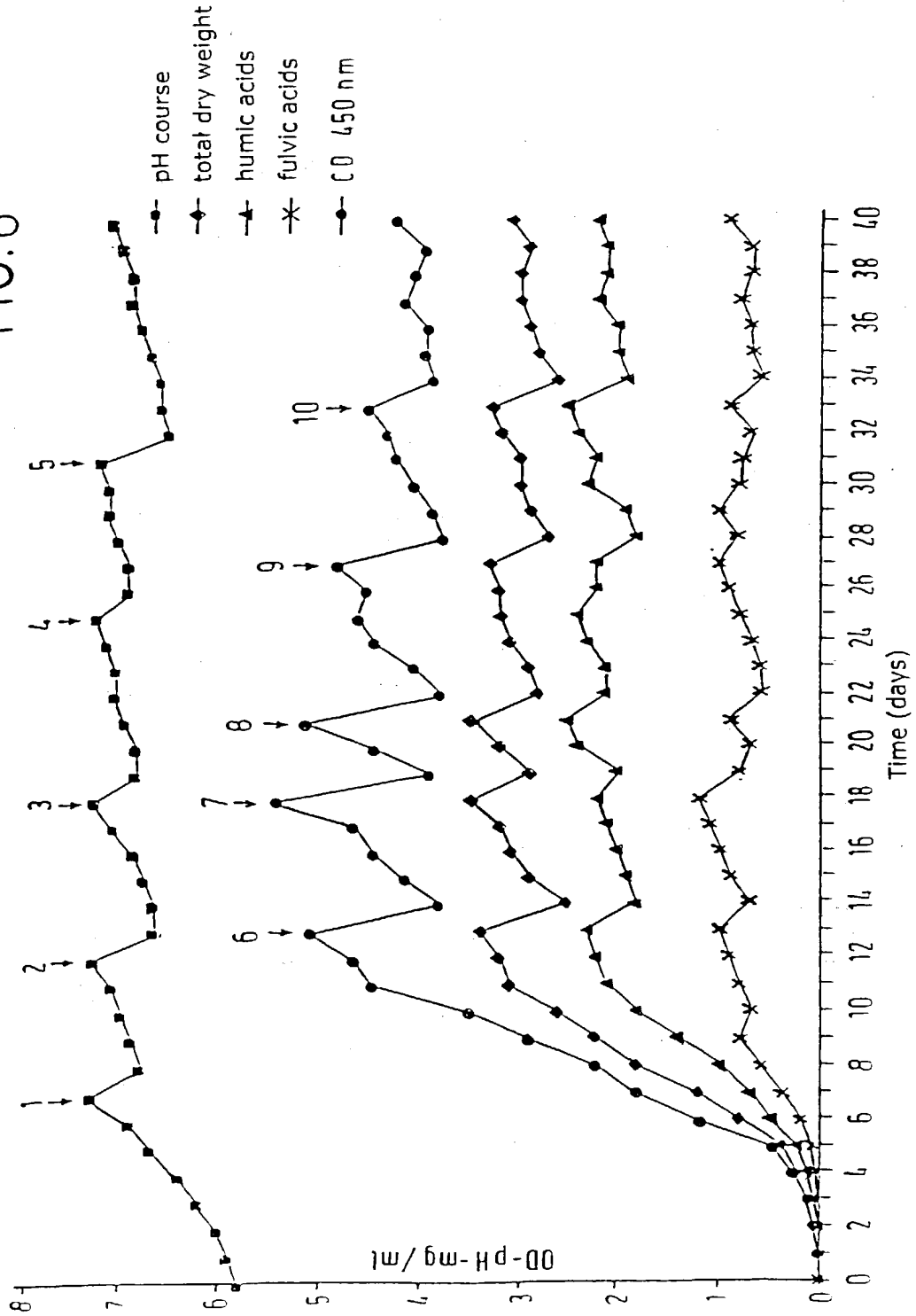


FIG.7

- 7 / 7 -

FIG. 8



(12) NACH DEM VERT ÜBER DIE INTERNATIONALE ZUSAMMENARBEIT AUF DEM GEBIET DES
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(71) Anmelder und

(72) Erfinder: HÖLKER, Udo [DE/DE]; Rottdornstr. 2,
D-53639 Königswinter-Rauschendorf (DE).

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(74) Anwälte: HELBING, Jörg usw.; Von Kreisler Selting
Werner, Postfach 10 22 41, D-50462 Köln (DE).

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LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX,
MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL,
TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

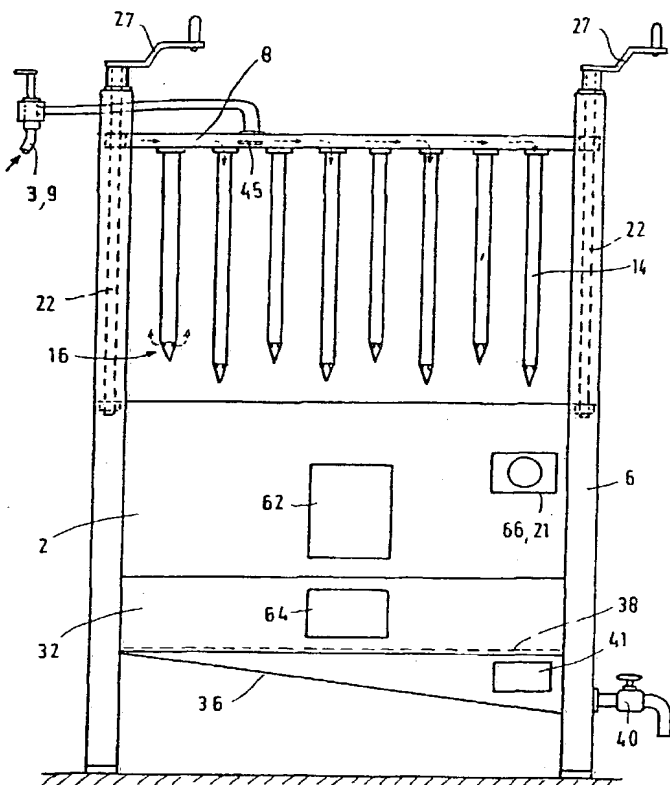
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(54) Title: BIOREACTOR FOR FERMENTING SOLIDS

(54) Bezeichnung: BIOREAKTOR ZUR FERMENTIERUNG VON FESTEN STOFFEN



(57) Abstract: The invention relates to a bioreactor for fermenting solid substrates, comprising a fermentation vessel (2), a device for feeding bioreactive substances and a nozzle arrangement in the fermentation vessel (2). The invention is characterized in that a nozzle arrangement (10, 20) consisting of a plurality of pipes (14, 24) which project into the reaction chamber (49) of the fermentation vessel (2) in parallel and which are provided with nozzles (16, 28) is situated in the fermentation vessel (2). The invention also relates to a method for aerobically fermenting solids. A reaction medium containing these solids is mixed using a compressed gas (48) which is guided into the reaction mixture from above.

(57) Zusammenfassung: Die Erfindung betrifft einen Bioreaktor zur Fermentierung fester Substrate mit einem Fermentationsbehälter (2), einer Zugabeeinrichtung für bioreaktive Stoffe und einer Düsenanordnung in dem Fermentationsbehälter (2), dadurch gekennzeichnet, dass eine Düsenanordnung (10, 20) aus einer Vielzahl von parallel in den Reaktionsraum (49) des Fermentationsbehälters (2) hineinragenden und mit Düsen (16, 28) versehenen Leitungsrohren (14, 24) in dem Fermentationsbehälter (2) angeordnet ist. Die Erfindung betrifft weiterhin ein Verfahren zur aeroben Fermentierung fester Stoffe, wobei ein diese festen Stoffe enthaltendes Reaktionsmedium durch von oben in das Reaktionsgemisch zugeführtes Druckgas (48) durchmischt wird.

WO 01/19954 A1



ATTORNEY DOCKET NO.: Kreisler 1101-KGB
020451 us/JH/ml

COMBINATION DECLARATION & POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name. I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

BIOREACTOR FOR FERMENTING SOLIDS

the specification of which was filed on March 13, 2002

as Application Serial No. 10/070,966, which is a 371 of PCT/EP00/08929, and

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)			Priority Claimed
<u>199 43 853.6</u>	<u>Germany</u>	<u>13 September 1999</u>	<input checked="" type="checkbox"/> yes <input type="checkbox"/> no
(Number)	(Country)	(Day/Month/Yr. Filed)	
<u> </u>	<u> </u>	<u> </u>	<input type="checkbox"/> yes <input type="checkbox"/> no
(Number)	(Country)	(Day/Month/Yr. Filed)	

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punished by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.



10070966-073102

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1/1
Kurt G. Briscoe, Reg. No. 33,141; William C. Gerstenzang, Reg. No. 27,552; Lorimer P. Brooks, Reg. No. 15,155; Bruce Londa, Reg. No. 33,531; Christa Hildebrand, Reg. No. 34,953; Howard C. Lee, Reg. No. 48,104; and Theodore Gottlieb, Reg. No. 42,597 all of 220 East 42nd Street, 30th Floor, New York, New York 10017; William R. Robinson, Reg. No. 27,224; Davy E. Zoneraich, Reg. No. 37,267; Mark A. Montana, Reg. No. 44,948 and Robert A. Hyde, Reg. No. 46,354, of 721 Route 202-206, Bridgewater, New Jersey 08807, my attorneys and/or agents with full power of substitution and revocation.

SEND CORRESPONDENCE TO:
NORRIS, McLAUGHLIN & MARCUS
220 EAST 42ND STREET - 30TH FLOOR
NEW YORK, NEW YORK 10017

DIRECT TELEPHONE CALLS TO:
KURT G. BRISCOE
(212) 808-0700

1-00

FULL NAME OF SOLE OR FIRST INVENTOR: <u>UDO HÖLKER</u>	
INVENTOR'S SIGNATURE: <u>[Signature]</u>	DATE: <u>24.06.02</u>
RESIDENCE: <u>Rotdornstr. 2, D-53639 Königswinter-Rauschendorf, Germany</u>	CITIZENSHIP: <u>German</u>

FULL NAME OF SECOND INVENTOR:	
INVENTOR'S SIGNATURE:	DATE:
RESIDENCE:	CITIZENSHIP:

FULL NAME OF THIRD INVENTOR:	
INVENTOR'S SIGNATURE:	DATE:
RESIDENCE:	CITIZENSHIP:

FULL NAME OF FOURTH INVENTOR:	
INVENTOR'S SIGNATURE:	DATE:
RESIDENCE:	CITIZENSHIP:

FULL NAME OF FIFTH INVENTOR:	
INVENTOR'S SIGNATURE:	DATE:
RESIDENCE:	CITIZENSHIP:

FULL NAME OF SIXTH INVENTOR:	
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RESIDENCE:	CITIZENSHIP:

FULL NAME OF SEVENTH INVENTOR:	
INVENTOR'S SIGNATURE:	DATE:
RESIDENCE:	CITIZENSHIP: